that was economic really more than anything.

Secondarily, the testing that we know of that other companies do, they do not share information. The gluten-free is an interesting industry. I think we all know each other but I don't think manufacturers like to share what is considered trade secrets, and that is considered possibly a trade secret. I have asked for a parts per million and I don't really know that I could evaluate it if I got the information, to be honest, to say one is better than another. So, a lot of what we do is really a compilation of, you know finding out. Sometimes somebody will say they have tested down to one part per million, and that is like a red flag right there, you know, because I know they don't test down that far for gluten. So, sometimes it just gives us more information about what the manufacturer may know. You know, even me as a lay person, I can see some red flags.

Then, I think to answer your question do they use consistently the same ones, no. Again, we also ask for food allergens as well so, besides

gluten where we may not look at it in terms of, you know, do you test down to this level and such, for allergens we look at it a lot differently, and part of it is just to see what lengths they are going to; how concerned are they; how scrupulous they are. So, some of it is qualitative in the results and how forthcoming they are about the information.

DR. KANE: I just want to make sure I understand. So, you do not ask the manufacturer what methodology they are using. You ask them for the parts per million but you don't know how they arrive at that, what test kit or methodology they are using. Is that correct?

MS. BERGER: No, if they have tested we have that certificate and that certificate says on there what they have used. But my ignorance would be I would look at it and I would have to call somebody else, like Steve in Nebraska, and say what does this mean to me? You know, I just don't have the knowledge to evaluate it critically.

DR. KANE: Thank you.

DR. PARK: Douglas Park, with FDA. Thank

you for your presentation. I do have some questions I would like to pose to you. One of them basically is a follow-up a little bit to what Dr. Kane was asking you. On your certification on your products do you do follow-up confirmations of your own when you receive a product, whether it is done on a random basis or whether it is done with each batch of the product that you receive?

MS. BERGER: When you say follow-up--

DR. PARK: Do you follow up testing?

MS. BERGER: Call the person that did the test?

DR. PARK: No, do you do follow-up testing your own? Within your own organization do you have a quality assurance/quality control program that provides you assurance that the product that you are receiving, your raw product, is in compliance and your in-line production is in compliance, as well as your final product?

MS. BERGER: We came about how we are doing it now somewhat deductively and not as analytical as I would like, and part of the fall

testing is not just for gluten but it is going to be for all of the allergenic proteins. Part of how we--I wouldn't say side-stepped, but part of how we tried to stay as scrupulous as we can to our own mixes, which are our products, is to get ingredients from people who only do one thing. So, the sorghum supplier we use, they only grow sorghum; they mill their own sorghum and they package their own sorghum. The rice we have to get all the way from California to get a dedicated farm that does those things. The products that we carry from other companies, which is somewhat separate because we don't have a lot of control over that--I don't do in-house testing on those at all but I do pay attention to customer complaints and we have had to follow-up with a voluntary recall and decide not to carry a product that the company still lists as gluten-free on their website today.

The plan is to do this fall in-house testing on our own ingredients. Even though we know inherently that they are gluten-free because they have been farmed as such, I still want that

level of assurance for myself--you never know--and test each thing to all the eight major allergens plus gluten in particular so that I can feel the best level of comfort that what we say we do, we do. But the problem really has been mostly cost and actually too my educational process to understanding because, again, I put my research hat on and actually had--I mean, I bugged University of Nebraska and called people that are dietitians that are friends of mine and just plugged them for questions.

So, we are not where I want to be and where I would like to be and comfortable to be to give a true sense of self. So, we are very clear with the consumer, particularly the food allergic consumer. Here is what we do; here is how we do it. We challenge you at any time, please call the manufacturer; please let us know if something has changed; please let us know if you ever have an issue with us. And, we do testing; we take ingredient lot numbers and then we take a mix and we make a lot number out of that, and we will go

back to every single ingredient and check, even though we do a quarterly, and just second-guess the supplier. So, yes and no.

DR. PARK: Do you anticipate setting up in your process a HACCP program which would be then also tied in with an in-house quality assurance/quality control program?

MS. BERGER: Definitely. That is, again, the goal with the ELISA testing, not only to have fail-safe mechanisms to prevent but, if they should occur, how would we handle it and, actually most importantly to me is how do we let the customer know? Because we are a national mail order, we go overseas and how do I let this person know? So, we have a database that can tell us a customer has purchased a product but we don't know what lot number it has come from to re-reference it, and how do you communicate to people who don't necessarily have e-mail; you don't have a phone number sometimes, and how to do it adequately? So, we have discussed with food allergy and anaphylactic networks to help us. Then, being part of these

e-mail news groups you can kind of get the word out. I mean, part of how we did the pasta was to politely let everybody know that our comfort level exists such that we would like to retract the product without trying to inflame the company.

DR. PARK: In the meantime, do you envision using an outside organization to do your testing for you on a regular basis--

MS. BERGER: Yes, in the fall.

DR. PARK: --and not necessarily quarterly--

MS. BERGER: No, I want to do quarterly--

DR. PARK: --I am talking about daily?

MS. BERGER: I want to do quarterly. I would feel more comfortable with quarterly because we do change suppliers regularly. You know, we will have to change a rice supplier because they are now doing something besides rice, or whatever. Maybe this is overkill but I would like to test every batch in-house that we do. Then I would like to use an outside independent lab by University of Nebraska, which is what I have chosen so far, to

double check my procedures and have it more honest. And, part of the celiac branding that I am looking forward to is, again, an outside body like the kosher certification that comes in and says you are doing what you are supposed to be doing. They have more knowledge than you, and they see things that you may not see.

DR. PARK: Along that line, have you looked at the economic impact of doing that?

MS. BERGER: Yes. I have 53 mixes and if my husband stops me now we will stay at 53, but I figure 53 mixes times 80 per quarter. But I don't think that is unreasonable to provide the level of safety I want to provide considering what I want to do. And, I want customers to feel that, to the best we possibly can do it, it is as clean as they possibly can get.

DR. PARK: Thank you.

DR. LUCCIOLI: Stefano Luccioli, FDA. In your talk you referenced that you often look for grains in Europe, which I think is a very good idea since Europe has a much longer history of dealing

with celiac disease and I am sure that they have tackled a lot of these problems. What are things that you have learned that maybe the American market could improve on in milling their grains? For instance, I understand that countries such as Scandinavia produce oats without wheat so that is never a problem. Is it economically feasible to do that in this country?

MS. BERGER: I don't know if I am your bird of choice. I know more about the actual finished product than the raw ingredients. We do get raw ingredients from overseas. The corn starch that we get that only does corn can comes from overseas sometimes because it is not as prevalent in this country, that you can find corn where they are only growing corn and they are only milling corn.

As far as economics and their knowledge, I think they are ahead of us in a lot of ways. I am not really sure if it is just that--like Dr. Shar for an example that has been around for years. In the pharmacies for years you have been able to

purchase gluten-free products and it has been identified for you so the consumer would walk somewhere and they would go get their prescribed diet. It may be more limited. It may be not as appealing but they felt safe that somebody had given them what they needed to work around instead of setting them loose in the Giant to try to figure out for themselves this pile of paperwork that they have compiled.

I can't really speak intelligently. I mean, I can tell you off the top of my head but I can't really tell you intelligently. I know that there are a lot of manufacturers out there overseas that are trying to bring products in, and there is a huge industry. I get letters almost monthly asking me if I want to be an importer or the retailer in this industry for it.

DR. SCHNEEMAN: For some of those questions we might be able to come back to the general panel too if there we have time. Was there anything else from the panel?

[No response]

Great! Thank you very much. Our last presentation for this panel is Mr. Lee Tobin, who is the team leader for the Gluten-Free Bakehouse at Whole Foods Market.

Gluten-Free Bakehouse, Whole Foods Market

MR. TOBIN: Thank you. The last audience I spoke to I think was a little more challenging than the FDA. I did a cooking demonstration for some group of children at the GIG celiac camp in North Carolina, and the six year-olds sat in the front row.

[Laughter]

I can assure you that celiac children are very well behaved, especially when you feed them. I wanted to start out by thanking the FDA for inviting me and Whole Foods Market to participate in these meetings. I would like to thank Jill Kuzo who is our research and consumer relations coordinator at the Bakehouse and Joe Dixon from Whole Foods quality standards offices, both of whom helped to prepare this presentation.

The Whole Foods Market Gluten-Free

Bakehouse is a dedicated facility that grew from a dedicated production run which I developed in one of our in-store bakeries after my diagnosis with celiac disease in '96.

We opened our new bakehouse--it is a fairly new facility, we opened less than a year ago in Morrisville, North Carolina last October. We produce 27 different baked goods from scratch. We are packaging everything in pallet size quantities and distributing them to approximately 170 of our stores throughout the U.S.

In this presentation I will be covering sections A, B and C of the Federal Register Notice; discuss our framework for defining gluten-free; and the Good Manufacturing Practices and the analytical methods we use to ensure that our products meet these standards.

Our stake in decisions involving gluten-free labeling is three-fold. Our primary concern is the confidence of our customers. They trust that an item labeled gluten-free meets their expectations. Secondly, it is consistency. We

need to assure that other products labeled gluten-free are set to the same or similar standards as those we have set for ourselves. Last, any standards set by the FDA of labeling of gluten-free products will necessarily impact our production and the measures we take to exclude gluten.

There are three methods we use to support our claim of gluten-free status. We gather testaments from ingredient suppliers. We perform ELISA testing on our ingredients and finished products, and we dedicate our entire facility to production of gluten-free with the exclusion of gluten from the premises. In the future we would consider participating in a third-party certification program, one that follows FDA guidelines, perhaps modeled after kosher or organic certification which Whole Foods has considerable experience with.

In addition to screening our ingredients for gluten, we also exclude artificial colors, flavors and preservatives. In most cases Whole

Foods Market support of minimally processed ingredients has made searching for gluten-free ingredients easier. Virtually all of our ingredients, with the exception of extracts and emulsions, are inherently gluten-free and not rendered gluten-free through processing.

We made a concerted effort to educate our suppliers and manufacturers about hidden gluten and sources of gluten cross contamination issues with shared lines or concurrent production. Once we have determined that each ingredient is gluten-free we gather statements from suppliers that express understanding of the term gluten-free and give assurance that their ingredients are free of gluten.

In addition, our facility employs a lot tracking system. We record lot numbers of each ingredient used in each batch and each recipe produced. This system traces all ingredients used from their origin to each batch of our finished product and allows us to initiate recall procedures as a safeguard should gluten, or any other form of

contamination, be discovered in our ingredients.

This screen illustrates points in the processing of ingredients that farmers, manufacturers and suppliers should be aware of to prevent gluten contamination. It refers mainly to grain and flour production which are higher risk ingredients but could be applied to other ingredients as well.

Crops should not be rotated with any form of wheat, rye or barley and should be harvested, stored, transported, milled and packaged with gluten-free equipment or in a gluten-free environment. We have always excluded oats from our production due to concerns about cross contamination, and we will continue to do so until the safety of oats can be consistently confirmed through the process.

Gluten testing--testing protocols are challenging to establish because there is no one method we can use to test 100 percent of our products. Samples must be taken at key points in the process and the number of samples needed

depends on the level of risk of contamination. We use the BioKits rapid gluten test from Tepnel Biosystems. It costs about $16 per test. This is a qualitative stick test that detects the presence of gliadin as low as 50 parts per million and yields results in about five minutes. We chose this test because it is fairly simple to use.

All testing is done at the bakehouse by our quality control person. We looked into sending out samples to a lab for testing but, as Jane mentioned, the costs are prohibitive. We currently test all new ingredients and a new source of an existing ingredient if we are to change suppliers since this is the best point in the process to exclude gluten from our bakehouse.

We also test random samples of finished products for additional verification. Finished products need to be sampled carefully so that particulates in the sample do not obscure test results, which has required us to do additional tests when we have had inconclusive results. Our sampling plan continues to evolve as we grow and

increase our production volumes.

We are in the process of developing a HACCP program which will include screening for gluten as a critical control point. With this program we intend to test samples from each lot of all high risk ingredients coming into the bakehouse and one finished product sample from each of our production runs. This testing program could be part of a possible third-party certification program, as I mentioned earlier.

Our decision to dedicate our entire bakehouse and work staff exclusively to gluten-free production was made primarily on the basis of minimizing the risk of contamination. Facilities that process wheat or gluten ingredients will always run a higher risk if they also choose to process gluten-free products.

This screen illustrates levels of dedication that food processors can use to reduce risks. Some dedicate production run and clean equipment thoroughly between runs. Some dedicate certain pieces of equipment or an entire line of

equipment, and some dedicate their whole facility to gluten-free production. Each higher level eliminates more points of potential contamination.

Cross contamination within our facility--there are some steps that we take to reduce contamination risks at the bakehouse and at our stores as well. We provide full uniforms for all of our employees and replace them daily. We also provide them with shoes and aprons. The aprons are worn over the uniforms but remain in the work area during work breaks as a protective barrier for any gluten that might be on their clothing. Visitors to the bakehouse are supplied with lab coats if they enter our production areas. We share our building with a warehouse that the company runs and there are several employees; people are coming in an out so we require those lab coats. We also signage throughout the facility to alert anyone who is coming in and our employees as well that it is a gluten-free production area and we need to exclude wheat, rye, oats and barley.

We employ strict cleaning procedures

between production runs to prevent any allergen cross contamination. I should mention that we are not an allergen-free bakehouse. We use dairy, soy, nuts and some of the other allergens but we do not promote our products as being allergen-free, just gluten-free.

At the store level we have initiated training programs that stress cross contamination issues with gluten and other allergens when employees in our store are either serving or sampling our products. We feel that education of all our employees is key to reducing potentials for cross contamination.

Any standards that are set for gluten-free labeling must meet the expectations of our customers. Customers must have the confidence that food labeled gluten-free is safe for them to consume and will not make them ill. In addition, standards must be feasible for producers to meet. Feasibility is determined by relative difficulty to implement, reliability of results and, of course, the cost. There also needs to be consistency in

definitions so that fair comparisons between brands of products can be made, and these definitions must be easy for the general public to understand. Education of consumers, manufacturers and lawmakers is essential to create a label that easily distinguishes gluten-free foods for celiacs who are medically required to be on a gluten-free diet. Thank you.

[Applause]

Questions and Answers

DR. SCHNEEMAN: First, why don't we take some time to see if there are some specific questions for Mr. Tobin, and then we will have time I think for more questions to be addressed to the panel speakers. So, do we have some specific questions?

DR. PARK: Thank you. This is Douglas Park, with FDA. You indicated, and correct me if I am wrong, that for many of your raw products you obtain certification that they are gluten-free. Is that correct?

MR. TOBIN: We obtain letters from our

suppliers and manufacturers claiming gluten-free status, yes.

DR. PARK: And I will ask you the same question that I asked the previous speaker, do you confirm in your own testing in your own laboratory that they are, in fact, gluten-free?

MR. TOBIN: Yes, we have done a limited amount of testing to confirm that. Our intention is to ramp up our testing once we have our HACCP program developed.

DR. SCHNEEMAN: Excuse me, let me interject. Do you have a certain expectation of what kind of test they would use in order to certify to you that they are gluten-free?

MR. TOBIN: No, we have not questioned our suppliers whether they have used testing procedures.

DR. PARK: That is a good follow-up question. You indicated that you are developing a HACCP program. Would you share with us a little bit your initial ideas as to what you would consider an appropriate HACCP program for a

gluten-free plant?

MR. TOBIN: I can't give you specifics because it is still under development and I am not the person who is working on it. We actually run two bakehouses. Our gluten-free facility is umbrella'd under an existing bakehouse that has been in operation for a number of years. It is located a quarter mile away and we have a quality control person who does work for both of our bakehouses and is working on developing that program. But the gluten testing will be a significant part of that program.

DR. PARK: In your dedicated facility you prepare only products that fall under your gluten-free assurance and label? Is that correct?

MR. TOBIN: That is correct. All of the wheat-based products are made at our other bakehouse.

DR. PARK: What about other cereals, other than just wheat-based?

MR. TOBIN: I am not sure I understand.

DR. PARK: Well, we are concerned not only

just with wheat; we are concerned with oats and some of the hybrids and specialty grains. Are those brought into that gluten-free area?

MR. TOBIN: No, not at all. Our warehouse that shares the facility primarily stores equipment, coffee and drinking water, firewood, pallets, that sort of thing. There is nothing stored on the premises, or processed.

DR. PARK: Those are all my questions.

DR. LUCCIOLI: Stefano Luccioli, FDA. I just wanted to follow-up. Did you define what you consider gluten-free? Do you consider oats in that definition?

MR. TOBIN: Not at this time, to deal with the cross contamination issues.

DR. LUCCIOLI: So, basically what all the other presenters have said, that is what your definition of gluten-free is?

MR. TOBIN: For the most part, yes.

DR. SCHNEEMAN: I was going to suggest why don't we have the panel come up? Don, go ahead and ask your question and then we can raise other

questions for other speakers. Go ahead, Don.

DR. ZINK: Don Zink, with FDA. Have you had situations where a supplier has certified that they are gluten-free and you have tested it and found it to contain gluten? And, have you had a situation where your raw materials passed muster but then, when you produced the finished product and ran the test, you had a positive gluten? I asked this question earlier. I am just trying to get a feel for the synchrony of claims of gluten-free with the actual test, as well as possible lot sampling and non-random distribution of contamination issues.

MR. TOBIN: To the first part of your question, we have not had any of our own in-house testing that conflicted a supplier's claim of an ingredient being gluten-free. With the finished product, we had a couple of tests that were inconclusive due to our inexperience with the testing method. The samples were shaken a little too much and rendered the inconclusive results, and we retested the samples of the same product and

that was confirmed gluten-free to greater than 50 parts per million.

Questions the FDA Panel

DR. SCHNEEMAN: Can the panel come up? Thank you very much. Stefano, you had a question of the last speaker about import, and I am wondering if Miss DeMarchi might be able to address the nature of the question that you had. So, I was thinking you might want to restate the nature of the question you were getting at. We will give you a microphone to respond.

DR. LUCCIOLI: Yes, Stefano Luccioli. I guess as a follow-up I have a question about oats. Why do we have a problem with oats contaminated with wheat? Is there just not demand for gluten-free oats, and why maybe in other countries are they able to supply these grains? Obviously, maybe it is an economic issue.

MS. DEMARCHI: This is Jane DeMarchi from the North American Millers' Association. I think I would caution you about cross contamination. Although wheat contamination may be our greatest

problem in the U.S. and Canada with oats, rye and barley are the grains of concern in Europe. I think in Ireland there are problems with rye contamination. I don't know as much about Scandinavian countries. I think that they may have a greater level of purity but I am not totally sure about that.

In reference to an earlier question you had asked about products coming from Europe, and I don't know if this is true but if they have a different type of identity preservation system that is used on grains in Europe, in part because of concerns about biotechnology, that may impact the level of grain mixture not just in oats but in other grains in the grain system in Europe.

MS. BERGER: Just in addition, as a manufacturer, if we were to find for an example a source that we felt to be clean, like I have heard McKane's oats hypothetically has been touted as clean and, yet, there was another one that disputed it--I think right now, if we were to take oats in, because there is so much in the understanding of

what is gluten-free to the community, we would basically be discrediting ourselves as a gluten-free supplier because there controversy is so great about it that even if we were to obtain it, until the definition is laid out that everybody is in consensus, I think everybody is very cautious to want to even introduce it anyway.

DR. LUCCIOLI: That is a good point. That is my feeling, that the demand, since you are going to have to include it in gluten-free anyway, then maybe there is not the demand to find a more purified form.

MR. GILLIAM: I totally concur with that statement. There is a real hesitancy within our company to put something in a package and put it in the marketplace and be labeled and in whatever percent, 50 percent of the celiacs' minds it is now being possibly tainted. We are just staying away from that.

DR. SCHNEEMAN: Please identify yourself.

MR. TOBIN: Lee Tobin, from Whole Foods Market. I just wanted to add that I agree with

what these folks are saying. We were approached about eight months ago by a woman representing a group of farmers, I believe in Montana, who were interested in growing dedicated gluten-free oats. I don't know how far along they are in the process of bringing a product to market, but we would certainly be interested if they could confirm gluten-free status of the products.

DR. SCHNEEMAN: Great! Thank you. Felicia?

MS. SATCHELL: Felicia Satchell, FDA. My question is for Mr. Tobin. I just want to make sure I am clear on your testing, and you are testing at 50 parts per million?

MR. TOBIN: That is correct.

MS. SATCHELL: So, for products that are below 50 parts per million, they proceed through production and distribution channels. Do you have any type of system set up where you get adverse event reports? Have you gotten any? Do you have a system that allows you to trace back to any particular product that might have been the subject

of an adverse event?

MR. TOBIN: Good question. Yes, we do get a tremendous amount of feedback from our customers, mostly through e-mail. We have someone working full time answering e-mail for us. We get phone calls as well. We have been growing out our product region by region and most of the issues we get, phone calls or e-mails we get from customers, are from customers who are new to our products as we have just rolled out to their local store. I would say all cases of customers claiming to have a reaction, we have not found that to be the case. They were either not diagnosed with celiac--we sell to customers who are celiac and customers who are on a gluten-free diet for whatever reasons, and some of them have various reactions, maybe caused by consuming gluten or consuming something else. We have never been able to confirm any contamination with our products. As I mentioned, we do have lot numbers. We track lot numbers with ingredients and we date code all of our products so when they are at the store and a customer has a

complaint or an issue with a specific product, we can track the date of production. We have actually had samples sent back to us and we have sent them out for testing, and we have not had any issues, no confirmed issues.

MS. SATCHELL: Thank you.

DR. KANE: Rhonda Kane, FDA. Lee, just to follow-up about your definition of gluten-free specifically, could you list the different grains that you exclude, and do they include spelt and kamut and triticale? Could you just state what the grains are that you do not use?

MR. TOBIN: Sure. For the most part, what everyone else had mentioned. We exclude all varieties of wheat. We are using sorghum flour, soy flour, rice flour of course, tapioca starch, potato starch, bean flours, kimwa flour and buckwheat and millet in their whole grain form. I think that is everything, and there are other grains that we would consider to be gluten-free that we are not using currently.

DR. KANE: You didn't mention rice.

MR. TOBIN: Yes, we use rice, quite a bit of it.

DR. LUCCIOLI: Stefano Luccioli. How about corn?

MR. TOBIN: Oh, yes, corn. Absolutely. We use corn meal in our cornbread and corn starch as well.

DR. KANE: Rhonda Kane, FDA. In the different grains that you use, do you ask the suppliers if they are dedicated, or do you only deal with dedicated suppliers? Or, is it up to them but you ask them to provide some sort of certification that their product is gluten-free? What type of suppliers do you deal with, with these other grains?

MR. TOBIN: We deal with a variety of suppliers. Bob's Red Mill is actually one of our suppliers. Most of our flours are brought in pallet size quantities, but we do ask for letters of certification, and we don't specifically ask for their testing methods, no.

DR. KANE: And they are not all dedicated

facilities? Correct? Is that true?

MR. TOBIN: The millers are, yes.

DR. KANE: The millers?

MR. TOBIN: They claim to be.

DR. SCHNEEMAN: I am going to ask the panel if there are just some last questions that you might have.

DR. PARK: Douglas Park, FDA. The question which just came across my mind is, is there such a thing as a gluten-free alcoholic beverage, such as beer and that type of thing? If so, what grains are used in those?

MS. BERGER: Jay Berger. Yes, there are two. One is using sorghum and I have forgotten what the other is--honey. One is a honey lager. So, yes. There has been a big interest. I don't know how it fares, to be honest, but there has been a big interest.

DR. SCHNEEMAN: Felicia, did you have a last question?

MS. DEMARCHI: Just as a follow-up to the oats discussion also, I think the demand of

interest to purchase is tremendous. I think the hesitation is, okay, when is it going to be considered safe enough that we all can bring it in comfortably and feel confident that we are providing something that isn't contradictory to what they are hearing otherwise.

MR. GILLIAM: Dennis Gilliam, Bob's Red Mill. Tangential to that, I would say if oats are determined to be safe or found to be uncontaminated, being in marketing, I have a sense that taking out full page ads in "Living Without" and other publications similar to that, it would take a six-month campaign of full-page ads to educate the celiac community to the fact that they are, in fact, acceptable.

DR. SCHNEEMAN: I think I am going to close this morning's session. I want to start by thanking our panel. These have been excellent presentations. I appreciate, number one, your sticking to the time but, number two, really focusing in on those questions and issues that FDA had put in the Federal Register. I think you have

provided us a wealth of information and your responsiveness to the questions has been very helpful as well. We are going to take our first break. I know many of you are probably anxiously waiting for that. It is 11:15.

There are restrooms. As you leave the auditorium, if you head back toward where you came in, there are restrooms along that corridor. Again, I would remind you that if you do leave the facility to go out to Cafe Wiley you will have to come back in through security. So, just keep that in mind if you are coming back in. Also, I would remind some of you who would like to add to the public comment period this afternoon, it you would like to do that please be sure and pre-register for that with the registration desk that is out in the foyer. So, with that, we will take a 15-minute break and we will reconvene promptly at 11:30. Thank you.

[Brief recess]

DR. SCHNEEMAN: I think it is going to be time to reconvene. Knowing that we have a lot to

cover in a short amount of time, I want to keep us on time as much as possible. The next panel, of course, has the challenge that they are the panel between you and lunch so I know they will want to make sure we keep to the schedule here. We are going to do a little bit of a switch. The panel members can sit in the front row for the presentations but then we will want our speakers to actually be in the front for the question period.

Our next section of the agenda is to address questions around the gluten detection analytical methods. We have two speakers for this particular section. Our first speaker is going to be Dr. William Hurkman, who is a plant physiologist with the Pacific Western Regional Office of the Agriculture Research Service of the U.S. Department of Agriculture.

Gluten Detection Analytical Methods

Detection of Cereal Proteins and DNA Using MS, ELISA and PCR

DR. HURKMAN: Well, thanks for inviting me here. It has been a pleasure listening to the

presentations this morning, and my presentation will be a little bit different. I am Bill Hurkman, from Western Regional Research Center in Albany, California, and my topic will be detection of cereal proteins and DNA using mass spectrometry, ELISA and PCR.

DR. SCHNEEMAN: Excuse me, Bill, could you move the microphone?

DR. HURKMAN: Is this better? I always have a problem standing close. So, I will take a different approach. I am a plant physiologist and I like to see how things work and take them apart. I like to look at assays and understand what is going on.

So first of all I thought, not to insult the audience but I thought I would start out defining some of these terms because they can be confusing. Wheat flour, when you combine it with water, forms a dough, as you all know, and it makes many nice things, some of which you may have eaten for breakfast this morning. If you knead it with excess of water you can get rid of the starch

granules and you are left only with the protein portion, and that is called gluten. Now, gluten consists of two protein groups, the gliadins and the glutenins, and these can be separated from each other by mixtures of water and alcohol.

So, that is all well and good but then you come across this term prolamins, and gliadins, and glutenins are also called prolamins, and together they make up gluten. So, it can be slightly confusing. Then to add to this, there are prolamins in other cereals besides wheat. I am going to focus today on the gliadins, so the wheat prolamins are called gliadins. In barley they are called hordeins; in rye, secalins; and in oat, avenins. So, I thought with that introduction, maybe as I mix these terms I won't lose you; I will try not to do that.

The topic of today's talk is detection methods, and there are several analytical tools available for detecting the presence of cereal prolamins in food products. Today I will just cover three of them. They are the ones that are

used principally. They are mass spectrometry, monoclonal antibodies and polymerase chain reaction, abbreviated as PCR almost by everyone.

Now, the first topic I am going to cover is the use of mass spectrometry for protein identification. The schema you see here is the one I use in my laboratory for identifying the many proteins present in wheat endosperm. Essentially, what you are doing is isolating proteins from the seed, and we focus on endosperm so we isolate endosperm, extract the proteins and run them in a two-dimensional gel so the net result is a 2D gel.

Now, what that is, it is a separation of proteins in a first dimension gel by charge, and then that gel is laid on top of a second gel--the rectangle you see on the screen there--which separates them by size. Once we have done this, we visualize the proteins with a blue stain. That is why the protein spots are blue. Then we scan the gels to get a digital image.

This digital image is used to match spots between different gels, for example, for comparing

different samples grown under different conditions. Once this gel is scanned, we then cut out the spots and we add trypsin to the spots to break them into fragments so that the mass spectrometer can handle them. The fragments are created by an XY robot. In this case we have one made by Digest Pro. Then these fragments are analyzed by the mass spectrometer which is a rather large instrument, about 8 ft. by 3 ft. and about as high as the podium.

What you end up with is data like this. To the uninitiated that doesn't look like much, but what that represents is the size of these fragments in their amino acid sequence. With that information, you plug it into a computer and check databases and you can come out with the identity of the proteins. This has really been a marvelous piece of equipment in my research. I always like to tell the story that when I identified my first protein it took two years. Now we can do 50 a day using this technology.

Here is an example of two fractions of

proteins from wheat endosperm, the gluten proteins and the albumins and globulins. What you see is that there is a whole bunch of proteins in these fractions. There are several hundred here and over a thousand proteins in this fraction. What we have attempted to do using mass spectrometry is to identify all these proteins.

On the slide I haven't done that but since this meeting is basically to talk about wheat allergen proteins, I thought I would show a few on the slides. The gluten proteins contain the omega, alpha, gamma gliadins and the high and low molecular weight glutenins. So, that is quite a few proteins that celiac patients can be allergic to or react to.

In the albumin and globulin fraction I have just labeled areas in the gel where these other known allergens, IgE allergen proteins are located. They include the serpins, glyceryl aldehyde 3-phosphate dehydrogenase, the GAPDH on the slide, peroxidase and also alpha trypsin inhibitors of which there is a large number in the

low molecular weight region of the gel. So, this technology is wonderful in that, if you can separate the proteins either on a gel or using HPLC, you can identify and separate specific proteins out of all the proteins present in wheat endosperm.

Advantages of the mass spectrometer identifying proteins are that, as you saw in the previous slides, it can separate and visualize many proteins at once. Protein identification is relatively rapid. Proteins can be quantified. Another useful thing about this technology is that proteins that you separate can be used to make antibodies against specific proteins of interest. One thing I didn't point out on this slide is that you can use this technology also in combination with antibodies and Western Blots to identify, for example, wheat allergy proteins.

The limitation to this method is that you can only visualize the most abundant proteins, but this can be overcome to some extent by fractionation, which I showed on the previous slide

where I used KCl to separate the two protein fractions. Also, the method is technically demanding. You need skilled people in your laboratory to do the protein separations and the mass spectrometry. Also, the equipment and the associated software is quite expensive.

Another one you have heard a lot about today is the ELISA, and that is short for enzyme-linked immunosorbant assay. This assay takes advantage of the ability of antibodies to recognize and bind to specific proteins which are termed antigens. It is more rapid and less expensive than mass spectrometry and is highly specific through the use of monoclonal antibodies.

On this slide is an outline of how monoclonal antibodies are made. First, the antigen is injected into a mouse to stimulate the production of antibodies. The antibodies are made in specific cells in the spleen called lymphocytes. These then are isolated and fused with myeloma cells to produce antibody-producing hybrid cells.

If you look at the slide you can see that

the little lines on each of the cells are different. What that is showing is that each of these cells make different antibodies. So, you can screen for the antibody of interest, and once you find that cell line you can clone it to produce unlimited amount of antibody for future studies.

What do we do with this antibody? We use it in what is called a sandwich ELISA. This is done in 96 well plates. The blue cylinder there is representative of one well and a plate. The first thing that needs to be done is to bind the monoclonal antibody to the wells of the plate. Then you add the sample and you also compare the sample with the standard so you can quantify the results. You incubate for a time and wash the sample to remove excess antigens. Then you add a conjugate. Now, what a conjugate is, it is another antibody that recognizes the antigen that is connected to an enzyme. Later I will tell you what that is about. Then you again wash it so that you have just your antigen in a sandwich. That is where the name comes from. It is a sandwich

between an antibody and a plate and an antibody connected to an enzyme. Then you add the substrate, and what happens is that the conjugate has the enzyme which utilizes the substrate and alters its color. After a certain time you stop the reaction with a stopping reagent and the color that is in the well is correlated with how much of the antigen is present. So, I thought it is an interesting assay and it is nice to know how these things work.

With respect to gluten proteins, what has become available in recent years is the R5 monoclonal antibody. This was produced by Mendez and his collaborators in Spain, and the reference is at the bottom of the slide. This antibody detects gliadins, hordeins and secalins but not the avenins. You might wonder how is this possible.

Well, the way this works is that the antibody recognizes the five amino acid sequence, QQPFP, and for those who aren't familiar with the shorthand, it is at the bottom of the slide and it stands for

glutamine-glutamine-proline-phenylalanine-proline. If you look at this sequence, this is a sequence of a wheat gliadin and I said that it is a prolamin because it has a lot of glutamines and prolines. If you look in there you see a whole lot of Ps and Qs so that is where that name comes from.

What you will see in this wheat gliadin is that that five amino acid peptide is in there 14 times. So, that is why this assay works because processing it this peptide gets fragmented and there are 14 chances that you can detect this particular protein using the antibody. It is a pretty cool method. Now, this sequence is found also in the hordeins and secalins but not avanins. So, that is why you have the specificity.

The advantage of the ELISA assay is that, as you saw, it detects wheat, barley and rye but not oat prolamins. It works well for a variety of processed and unprocessed foods. It is relatively rapid. The R-Biopharm assay literature says that it can accomplish this in an hour and 30 minutes--not too bad but not fast enough for

assembly line production. In a laboratory setting it is sensitive down to 1.5 parts per million, and it is available in several commercial kits.

Two of these kits were tested by 20 laboratories in a study that came out in a symposium proceedings by the Prolamin Working Group last year, and the conclusion was that it was rapid and reliable, and that statistically reproducibly it was quite good. This assay has been temporarily endorsed by the Committee on Methods of Analysis and Sampling, which is part of the Codex Alimentarius Commission. The reason it is temporary is that they would like to see more data; they would like to see more samples tested, and so forth. Anyway, it is a good start for that.

The disadvantage is that it does not distinguish between wheat, barley and rye so if you have all three contaminants in one sample all you know is that it is contaminated, but not with what and in what proportion.

Another issue is that it detects only gliadins in wheat. Some celiac patients also react

to high and low molecular weight glutenins. So, this may not be a major issue in ingredients that contain gliadins and glutenins but some, most notably starch, preparations don't always have gliadins associated with them and they have glutenins. So, it is something to be aware of.

The last assay I want to talk about is PCR amplification. Essentially, this is a method to amplify a specific segment of DNA to levels high enough to detect by assay. The reaction consists of three parts. The first is to denature or unwind the DNA template so you go from this helix to double strands. What this does, it allows the primers to anneal or bind to the DNA segment for which they are directed. Once the primer is annealed onto the DNA, then you can have the synthesis of the peptide of interest.

Now, what you need are forward and reverse primers, which is indicated here, to carry out this reaction, and when you want to extend these polymers you need DNA polymerase and nucleotide bases. You have probably heard of a thermal

cycler. It is how this assay is done. What it does, it cycles through this a number of times to increase the DNA to high enough levels to work with.

Now, what I did, I took from this publication by Sandbert et al. some data to show you how PCR works and why it is so specific. The red boxes indicate the forward primers; the blue boxes the reverse primers. This sequence, here, is specific to wheat gliadin. In other words, it is not found in rye, barley or oat. Similarly, this sequence is specific only to rye and is not found in wheat.

Again, just to show this for hordein, the same thing. Here is the specific sequence and this is the specific sequence for avenin. What this means is that, unlike the ELISA assay, you can tell which of the cereals are within your sample by doing this DNA detection.

Here is sort of a summary table showing how specific these primers are. The wheat primers detect only wheat, not rye, barley or oat and all

of these types of wheat make that selected DNA sequence. In other words, whether the sample contains winter wheat, spring wheat, durum wheat, spelt, kamut or triticale wheat, it detects that. The rye primer only detects rye; barley, only barley; and oat only detects oat.

So the advantage of PCR is that it is species specific. In any given sample you can tell whether or not it has any wheat, rye or barley within it. It is sensitive and rapid. It complements ELISA results. In other words, the disadvantage is that it detects DNA rather than protein. ELISA detects protein but if there is contamination, say, from rye the ELISA shows that this test is contaminated. The PCR probe with tell you that the contamination is due to rye. So, that is kind of a nice thing.

In summary, mass spectrometry is an excellent tool for protein identification. It is sensitive but not really rapid when you compare it to ELISA and PCR. It is expensive and technically demanding. The ELISA works well for a variety of

unprocessed and processed food. It is sensitive and rapid. The main advantage of PCR is that it is species specific, complements ELISA and it also is sensitive and rapid.

What I have to say is that, as a user of mass spectrometry, I think that if you want to confirm absolutely results of ELISA it is the way to go. So, that is all I have to say today.

[Applause]

Questions and Answers

DR. SCHNEEMAN: We will take some questions right now from the panel. Also, if some of the other speakers are trying to get some questions, please alert one of the panel members that you have a question. I think it is the easiest way to get recognized. We know Doug will have questions.

DR. PARK: Douglas Park, with FDA. Considering the viewpoint of the individual that is susceptible to gluten, is it crucial that you know exactly which allergenic protein is present?

DR. HURKMAN: I would say the answer to

that is no as long as they know that it is contaminated with wheat--

DR. PARK: Or one of the others.

DR. HURKMAN: --or one of the others, yes.

DR. PARK: So, then going the next step and saying if we try to set up a two-tier testing system, first with ELISA as you have presented here and then a confirmation with either PCR or mass spec., throwing away your bias for mass spec., which would you recommend would be a confirmation?

DR. HURKMAN: I think you answered that it is PCR because of its specificity and it is a lot less work than mass spec., for sure. But if you run into a question that you can't answer maybe mass spec. might be--

DR. PARK: Three-tier testing. Relative cost--I know you indicated that this is very expensive, and also relative time for both the mass spec. and the PCR. Could you elaborate on that a little bit?

DR. HURKMAN: I will try. Mass spectrometry, if you want to use a company that

provides that service, runs about $150 to $200 per sample. That means that you are preparing this sample and giving it to them, which can take some time. The samples that we prepare take roughly a day and then with the mass spectrometer you can do 50 samples in a 24-hour period. So, that is where I got that it is relatively slow and expensive.

On the ELISA, I can only tell you that a kit is roughly $300 for 42 assays and it takes about an hour and a half. I was asking Jupiter about that timing. That includes the pipetting of the sample into the wells through reading the final results.

For PCR I don't quite know. In our laboratory we often start PCR the night before and get the samples the next morning. We can do a 96-well plate and machines can do, of course, more than one plate. Its cost is mainly for kits. Again, they are roughly $200 or $300 for a kit that can keep you happy for about a week or two.

DR. KANE: Rhonda Kane, FDA. Thank you, Bill, for your presentation. On one of your slides

you mentioned that some ingredients have glutenins and not gliadins. Can you give me any examples where you would have a food ingredient that would not come with both proteins together?

DR. HURKMAN: We are doing a project looking at various manufactured starches, and those are what I am referring to. The starches are relatively free of protein, low protein content, and of the proteins that are there, they are glutenins and not gliadins. Along with other cellular proteins, they seem to be a magnet for cell proteins. They are like a non-specific column for endosperm proteins.

DR. KANE: Is this wheat protein that you are talking about?

DR. HURKMAN: Yes.

DR. KANE: That only has the glutenins and not the gliadins? Is that correct?

DR. HURKMAN: No, it has the glutenins--

DR. KANE: I am sorry, glutenins and not the gliadins?

DR. HURKMAN: And this is wheat starch.

DR. KANE: And it is wheat starch. I had another question about the ELISA method that was mentioned by Jay Berger, where she said that the University of Nebraska mentioned that hydrolyzed protein and fermented proteins require a special ELISA--

DR. HURKMAN: Right.

DR. KANE: --could you elaborate on that? What is so problematic about detecting those types of proteins?

DR. HURKMAN: What happens is that on processing with heat, whether wet or dry, the proteins denature and once they are denatured you can't solubilize and assay them. So, it is interesting. One objection I have to R5 monoclonal antibody is that it is patented and not anybody can do this so you have to run around and either create antibody yourself because they won't sell it to you and it only comes in a kit, or you figure out how to do it. In this case, it is very funny--I always try to find out how to do things and there is a patented solubilization buffer exactly for those

types of proteins that they either include in the kit or you buy separately. That kit contains agents that denature the proteins so that when they go into solution you can assay them. Of course, the ingredients are secret. But in one publication I read it includes beta mercapito ethanol and, of course, it would. I mean, that is a standard component for doing that sort of thing.

DR. KANE: I have one last question and that goes back to the starch. Do you know anything about modified food starch and how it may differ from just regular wheat starch--

DR, HURKMAN: No.

DR. KANE: --and if the proteins in that are--

DR. HURKMAN: No. Unfortunately, I haven't read up on that yet.

DR. KANE: Thank you.

DR. ZINK: Don Zink, with FDA. Is this R5 monoclonal antibody the one that is used in all of the commercial test kits now? What I am getting at is do these commercial test kits suffer from an

inability to detect glutenins?

DR. HURKMAN: You could ask Jupiter. He can shake his head. But my feeling to your answer is yes. Is that correct? I am not sure about that because the Mendez kit is from Spain and I have no idea how many kits--at least two or three use them and I don't know how many kits are out there. DR. YEUNG: This is Jupiter Yeung. Actually, I am going to talk about three different kinds of antibodies that are used in the commercial market. The Mendez kit uses a different polyclonal antibody made by themselves.

DR. SCHNEEMAN: It would probably make sense to go ahead and have Dr. Yeung give his presentation.

DR. ZINK: Actually, I have one more quick question. On the refined starch do you consistently have enough DNA there to make the PCR reliable?

DR. HURKMAN: That is a good question and I think the only way it could be answered is by someone that has done it. We have not done that in

our lab so I can't answer that. But it is a good question because it is not clear always that there will be enough DNA in your particular samples.

DR. SCHNEEMAN: Great! Thank you very much. Our next speaker in this section is going to be Dr. Jupiter Yeung, who is going to talk about some of the commercial test kits and methods.

Commercial Gluten Test Kits and Methods

DR. YEUNG: My name is Jupiter Yeung. I am the principal scientist with the Food Products Association.

There are three different test methods, as has been alluded to previously, and I am concentrating on just the commercial test kits based on the immunochemical methods, the ELISA and the lateral flow device that is the dipstick. The mechanism has been alluded to so I am going to skip it.

In the commercial world for the gluten test there are three different antibodies that are being used. The first one is the skerritt monoclonal antibody which recognizes the omega

gliadin. Although it is a monoclonal antibody, it has also been identified that this antibody, also recognizes the R5 gliadin. So, the reactivity towards three different varieties of grains are different, with the wheat being 100, rye 120 sensitivity and barley only 5. The monoclonal antibody has been available for a long time, since 1991, and is the AOAC approved official method, in '95.

The other kind of monoclonal antibody commonly being used is the Mendez R5 which recognizes the five amino acid sequence and that recognizes R5 beta, gamma and omega gliadins and they are supposedly equally sensitive to wheat, rye and barley. Both antibodies recognize raw and cooked prolamins.

Here are the commercial test kits available in the U.S. market. There are six companies. Neogen is a U.S. company. R-Biopharm is from Germany. Tepnel and Hallmark are from the U.K. Diffchamb is from Sweden and Morinaga, a new company currently just getting into the market, is

a Japanese company. Only the first one, the Neogen Alert is a qualitative test. The rest of the test kits are all quantitative.

The limit of detection and limit of quantitation and the range are all in ppm gliadin. So, if you want to convert it to gluten you have to X 2. Within this box you insert how many mcg/ml or ng/ml so I converted it to ppm to make it a little bit easier for everybody to compare. But recognize that for the Japanese one--actually, I should have two stars there because the reporting unit is different from anybody else. It is not just gliadin but, rather, a wheat protein. So, when you do the interpretation, they are not the same. If it is a quantitative analysis, once you look at the error kill, the error kill is from 1 ppm to 50 ppm detection limit.

The Tepnel and the Hallmark, although they are two different companies from the U.K., are essentially the same product because the owner, originally from Tepnel, developed all the test kits for Tepnel and now has his own company so they are

essentially the same. So, all the companies Neogen, R-Biopharm--they use the R5 monoclonal antibody. Tepnel and Hallmark and Diffchamb use the skerritt antibody. With the skerritt antibody, they always have two different analyses, the regular one and the sensitivity is 50 ppm of gliadin but they all have a high sensitivity protocol that includes Tepnel, Hallmark and Diffchamb.

The high sensitivity assay will give you 5 ppm of gliadin so, depending on which method you use, you have different sensitivity and this is how many replicates you have to do per sample. The cost is here. But recognize that for the cost you can not just divide it by how much money and how many samples you have to run. Every time you run an assay you have to run the standard curve; you have to run your quality control samples so one cannot just say because they charge you $15, it is $15 a sample. It is not that cheap actually. But then one has to also recognize that they spent hundreds and thousands of dollars to develop the

test kit so those are factored into your final product. Like the mass spec. you have an expensive instrument that you have to outlay.

Here, is the validation of the test kits. The RidaScreen was validated by the Ring trials of the Prolamin Working Group. For the BioKits and the HAVen one they used the AOAC method but original protocol was verified by the AOAC but not the test kit itself. All the test kits would have the validation data either internally or neutral site testing. If you ask the company, I think they will share with you--I don't know if they will share with everybody but I have all the validation data.

They have two different kinds. One uses the skerritt antibody, the other uses the R5 antibodies. So, the first one was validated actually by Sweden, the national food authority administration, the government agency. Morinaga was verified by the National Institutes of Health Sciences in Japan. I haven't seen the data yet but they are going to present in the AOAC meeting this

month. From what I heard, it satisfied the government.

Here is one of the ELISA tests we use. This is the standard curve we use, and in all the samples you compare the color intensity with the standard curve. But this is not exactly that all commercial kits use this color scheme so it is different and another company product will give you a different color scheme, but it is all based on comparison with your controls.

If you look at the lateral flow device, the dipstick, there are primarily two different companies providing it. The R-Biopharm gives you 10 ppm of gluten. This time it is gluten because that is what the company insert declared. The Tepnel uses 100 ppm of gluten. Although it is two different kinds, essentially they are the same sample; everything is the same except they don't have the controls for the home test kit.

The mechanism is exactly the same as the ELISA test. It is a sandwich mechanism. So, if you have the antibody labeled and the antibody

trapping in your sample in here and the control antibodies here when you dip into the sample, the sample containing gliadin, for example, will pull up and will bind with the antibody and will be trapped by another antibody for the test, and will give you a sandwich and will give you a color, and the control will be trapped here and give you a different color. So, it is essentially the same use. It will give you a few minutes for the reaction and for the whole thing to occur, but you have to wait until it dries up. So, in a total of about five minutes you will have your result.

So, if you have two lines it is positive according to this company's product, the control and the test line--so positive two lines, negative one line, and if there is no line it means something is wrong. Either your sample is not meant for this method. In fact, it happened. Then you have to do it again. Sometimes it is your problem; sometimes it is your sample's problem.

But most of the dipsticks are two lines. So, if you use a different company product--this

one actually is the Tepnel one--two lines is negative; three lines is positive. If it is only one line your sample is overloaded. So, one has to recognize which commercial dipstick you are using so your interpretation will be different.

But also recognize here when they say 10 ppm or 50 ppm, when you see the line it does not mean it is 10 ppm or 50 ppm. It can mean much less than that. And we also have data to show that even if the detection is 10 ppm, you can see it; 5 ppm, can see it, depending on how strong the line is. If you are color blind you may not see it, but if you are not you can see it.

So, there are different issues with the gluten test kits. Depending on which test kit you are using and depending on whether it is the skerritt antibody or the R5 antibody the reactivity is different and the extraction media is also different depending on methods. Some use 40 percent, some use 60 percent alcohol and some use cocktails and some don't; whether it is cooked or not; or if you don't know whether it is processed

or not processed. And there are also different standards being used in different kits. Most of the European test kits use the Prolamin Working Group standard now with the IRMM-480, but then the 480 is not commercially available yet to the market. Some use the AOAC standard. All the standards are not identical.

If you have a false positive and false negative in the ELISA, it is very, very difficult to confirm it because most of the alternative confirmatory methods, like mass spec. or PCR, are not available to most food companies, although one can use the antibody based Western Blot to confirm it but it is still the same mechanism.

Obviously, same as any other allergens or other contaminants, there are no official or no recognized sampling plans so random testing--what is random testing mean? I can get a positive sample and I can get a negative sample very easily if you know what you are doing.

Extraction media--there are different extraction media and cocktails, and also either

alcohol or the cocktail and in the cocktail some ingredients are not known, and some we know interact with the antigens and antibodies and the mechanism is the antigen/antibody reaction.

The cocktail is also known to interfere with some of the complex matrices and it is reported in the Prolamin Working Group proceedings. Another thing is that when samples have a high polyphenol, tannins or syrups, that kind of thing, your recovery tends to be very, very low if you don't take care of it. Some have additives in. Some people add fish gelatin to improve their recovery.

Here is some of the information that we know. Here is R-Biopharm data. Here is 40 percent and 60 percent ethanol, 40 percent ethanol here, and the cocktail here. One can see from the same sample, the same homogeneous sample, that it will give you all different numbers. Usually the cocktail will give you quite a bit higher number. But are these high numbers the true numbers or not? One has to ask the question. The previous speaker,

Bill, mentioned these 14 repeating units in the DNA sequence. So, is the antibody recognizing one unit or recognizing multiple units?

This is the PCR and it can differentiate or speciate in a different one but not for the antibody-based methods. For the partial hydrolysis from the syrup if you use the sandwich-based ELISA your recovery will be low so the Mendez group actually has an alternative method. Instead of a sandwich ELISA, they have a competitive ELISA to, so-called, better quantify the hydrolyzed product, and it has been published. But it still depends on whether you have access to the antibodies or not so it really doesn't help too much.

Reference material--IRMM and TLC in Europe have the 480 for wheat gliadin but, unfortunately, it is still not available. The nice thing about is it is fully characterized and also it is soluble. If you use the reference material, there are different kinds and they are not identical so what do you choose? Alternatively, you can buy from Sigma, you can buy from any other companies but

they are not the reference material and they are not identical either as far as a confirmation method goes, and I think the previous speaker, Bill, has already mentioned this and discussed it with you.

Here is the information for the three different methods. These numbers are the ELISA quantitation numbers. If you mass spec. you can see all the alfa, beta and gamma gliadins using the mass spec. method. You can use the immunoblot of the antibody here, at the R5 antibody gel, and it will tell you which proteins will be recognized. This is the PCR for two different samples. So, mass spec. and the PCR methods would be a good marker to identify the allergens.

For detection and speciation, the antibody-based method can give you a summation of all the different kinds of grains--wheat, rye and barley. But the PCR and the mass spec. will give you alternatives or speciate which grain may be involved. If Health Canada is going to require speciation of source labeling of the wheat for

celiacs, then we need an alternative method to tell whether it is wheat, rye, barley or oat. That is all for me now.

[Applause]

DR. SCHNEEMAN: Thank you very much. We will take some specific questions. Dr. Hurkman, do you want to come up to the front and we can have some specific questions for you right now and we can address the panel as a whole? Rhonda?

Questions and Answers

DR. KANE: Thank you, Jupiter. I had a question so I understand one of your slides. It is the fourth one in your series where you have gliadin antibody specificity, and you have wheat 100 percent. Is rye 120 or is it 20?

DR. YEUNG: It is 120 because the antibody was originally starting from rye so it is more reactive to rye than wheat.

DR. KANE: Can you explain what those percentages actually mean?

DR. YEUNG: It is the cross reactivity of the antibody. So, if you plot an inhibitory curve,

the IC50, then you know that rye is more reactive so it is more to the left than wheat and than barley.

DR. KANE: Thank you. I have just one more question. You mentioned, I thought, that there were three types of antibodies.

DR. YEUNG: Yes.

DR. KANE: What is the third?

DR. YEUNG: The third one is Morinaga's polyclonal antibody. They make it themselves. They did not get a license from any of the producers.

DR. KANE: So, that is not on that slide? Is that correct?

DR. YEUNG: It is not on the slide because Morinaga just came to the U.S. market and at this point in time they are trying to evaluate the test kits.

DR. KANE: Thank you, Jupiter.

DR. YEUNG: You are welcome.

DR. PARK: Doug Park, FDA. Thank you very much for a very comprehensive summary of the

methodology. I do have some questions and to some of the questions we already know the answer but I think we need to have that brought out. You pointed out and discussed briefly standards, the availability of standards and so forth. Would you address the composition of the standards that you listed and the potential availability to any organization that would want to have the standard for confirmation of their testing program?

DR. YEUNG: The reference material from NIST and IRMM, for that matter, too, they are the official reference material organization, and it is available to the whole world. It doesn't matter who buys it or where you buy it, it would be the same material, unlike if you buy it from Sigma; it would not be the same. All the NIST materials are available. The IRMM-480 are not officially available yet but 480 is an alcohol soluble protein and is meant for this kind of work. The NIST reference material, the different varieties of wheat, was not originally meant for allergens. So, for characterization it is different. You look at

how much percent protein, carbohydrate, minerals--all the basic quantitative data that you can get from NIST.

DR. PARK: Thank you.

DR. YEUNG: The reason why we always push on reference material, the same as FDA--we worked together for a long time, pushing for the reference material because if you use different reference materials you would get different numbers that you would generate, different numbers from your test kit, and if you used it for your quality control samples they would also give you a very, very different number than what you are going to see, and your recovery would be different because you use a different standard so you are comparing a little bit different--it is not the difference between oranges and apples but it is definitely a different species so you will get a different result.

DR. PARK: Thank you. Also, on your slide where you showed the various levels of quantitation, and so forth, the Tepnel method has

50 parts per million. Does that mean that is the lowest you can go with that or are there other options?

DR. YEUNG: The Tepnel one originally was based on the skerritt procedure, the AOAC official method, '95. Because it is the official method it has been used for a long time but for the last couple of years, with everybody looking at the Codex number pushing down to 20, they are pushing it down to 20 ppm, so-called, the high sensitivity procedure. Then they put in a factor of 2 and made it 10 ppm.

DR. PARK: You also mentioned the matrix effect of some of the extraction procedures, in particular the cocktail. Are there issues where that can be addressed as well?

DR. YEUNG: Issues have been reported but because we do not know exactly what is in the cocktail--we know it is there--and some companies use it so you can imagine what would be there but we do not know exactly what kind of issues there are. But in our hands, when we use the regular

procedure for testing and also when you add the cocktail in the numbers are different, substantially different. So, as a scientific organization, we probably want to take a closer look at these issues.

DR. PARK: You identified in your slide for instance milk but do you have that same effect with the cereal grains?

DR. YEUNG: I do not have experience in it but that has been reported by the European group in the 2004 proceedings.

DR. PARK: Was that the cocktail that was used for the validation study in Europe?

DR. YEUNG: That is right.

DR. PARK: And the data there suggests that it is fairly consistent and acceptable for those commodities that were tested.

DR. YEUNG: Are you talking about the Ring trial?

DR. PARK: The Ring trial.

DR. YEUNG: All the data reported from the Ring trial is consistent with all the other

multi-lab validation procedures.

DR. PARK: This is my last question, and you will never be able to answer it because it is one of the biggest challenges. You did point out the difficultly with sampling, sample preparation, and so forth. I would like you to just take a minute longer and point out some of the difficulties associated with sampling as it deals with potentially allergenic products, dealing with the raw product as well as the finished product.

DR. YEUNG: I think everybody is trying to look at FDA and trying to get a guidance document to see what FDA's guidance is as far as sampling is concerned. I know you guys had an extensive look at this and we were told before that the guidance document will come out soon. I don't know if it will be soon or not. But I think most people still want to have guidance from the regulatory agencies so what we do can reflect that the inspector who comes to the plant and takes a sample will be consistent with what a company would want to do. Of course, the problem of allergens is that the

contamination is heterogeneous and it is very sporadic, and also the sample would be very--you can have contamination at one portion of the process in-line or during the production cycle. So, sampling would be the biggest challenge. At the same time, because of the expense of the testing and the time involved with the testing, one cannot over-test during production.

DR. PARK: In your opinion, when you look at the analytical result which is a sum of errors associated with sampling, sample preparation and analytical, where is the largest error associated with those three categories?

DR. YEUNG: I think Whitaker presented and published in the journal of OAOC recently, in 2004, and if my memory serves me right, which doesn't happen very often, it is the sampling that gives you the highest variability, not the analytical method. Actually, the analytical procedures gives you the least uncertainty.

DR. HURKMAN: I think you asked a good question, and that is what about different

samples--flour versus process? To me, and the work that I do, that is the critical point. Unfortunately, what we find is that for every sample we do we optimize the extraction methods and I think it is going to take quite a lot of work to categorize samples and how to extract them for best results or reproducible results.

DR. LUCCIOLI: Stefano Luccioli, FDA. I guess with the availability of some confirmatory tests like PCR or mass spec., do we have preliminary data on false-negative rates for the ELISA kits or is that something that we just don't have data on? I mean, how many times can one of these tests be falsely negative and it has been confirmed with a PCR?

DR. HURKMAN: I don't have a full answer. The Ring study is the only study I know with that sort of data. Right? If it even has it, and this was published in the symposium proceedings. That is hard to get. I have a copy back at the lab. So, that hasn't been directly addressed and I think that is why the assay is temporarily recommended

rather than fully endorsed.

DR. LUCCIOLI: Thank you.

DR. PARK: I would like to comment on that, and I am going to put on my FDA hat. This is Doug Park. That is the reason why it is crucial that methods are validated because that is the type of data that you get from a validation study. The Ring trial is an example of that. The AOAC harmonized protocol is an example of that, where you have 95 percent confidence in the analytical result, and that addresses your false positives, false negatives.

DR. YEUNG: This is Jupiter Yeung. I think for the false positives and false negatives, from the Ring trial there is information--I don't have the exact data with me, but the challenge is on the actual sample. It would be very, very difficult to prove if it is a false positive or false negative from a real sample because all the test kits are meant for all food matrices. Obviously, everyone is going to spend a little bit of time in the lab. You know, it is not possible;

it is all matrix dependent.

DR. SCHNEEMAN: Do you have one last question?

DR. LUCCIOLI: Yes, a follow-up to that. I guess we could be able to get information on which matrices may be more difficult to detect, and so forth. So, I mean, some information. I don't know if it is available but I was just curious, I think Miss Berger talked about how she sent samples to labs, to Steve Taylor's lab and the University of Nebraska, and I am just curious to know what has been the lowest level of detection, parts per million, of a consumer complaint, if that data is available.

DR. YEUNG: If you are looking at the company claim for the limit of detection, the lowest one is the R-Biopharm RidaScreen, which is 1.5 ppm of gliadin. That is 3 ppm of gluten. That is the lowest one. The rest are all 10 ppm and gluten.

DR. SCHNEEMAN: Did you want to comment on that question? Identify yourself.

MS. BERGER: Jay Berger, Miss Roben's. I was just wondering, you know, as a manufacturer of the products trying to come up with the appropriate testing for celiac disease and allergens, will the summation of this discussion evolve into specific procedures, specific tests and how to go about doing them so that we can provide the best accuracy to the product for the consumer?

DR. SCHNEEMAN: FDA is certainly in the process of gathering as much information as we can. So, we can make decisions within what we are charged to do under the law. So, you know, at this point we are trying to learn where we can go with the information, and that is really the purpose of this particular session, to learn as much as possible so that we can then make some regulatory decisions.

I think at this point we will need to break for lunch. I do want to again thank our two speakers for excellent presentations.

[Applause]

We really do appreciate your looking at

the questions and issues we have and helping us get the information out that we need.

Again, we have an hour scheduled for the lunch break so I would like to see everyone back here at 1:35 for our next panel. The most convenient place is the Wiley Cafe but you will be exiting security. For those that are more adventuresome, certainly there are some areas in College Park that are possible too but just keep an eye to the time. Thank you.

[Whereupon at 12:35 p.m., the proceedings were adjourned for lunch, to reconvene at 1:35 p.m.]

A F T E R N O O N P R O C E E D I N G S

DR. SCHNEEMAN: Well, I think we had better get started. We are ready for our third panel and I do want to comment. I know we kept telling you that Cafe Wiley may be your best and simplest option, and I understand that there were some problems with the delivery and food service. We thought that we had sufficiently prepped them for having the public meeting and having the types of food that would be appropriate. So, I apologize if any of you were inconvenienced. We are going to follow-up to see where the gap was. It is embarrassing to us to have you here as our guests and not be able to offer the service that we thought we had prepared for. I hope that wasn't a hardship for anyone in particular and, please, accept our apology.

As I said, our next panel will be to focus on the consumer perspective on a gluten-free food labeling standard. We will follow exactly the same format that we have been following so far where we will have the three presentations and some

questioning of each of the presentations and then, hopefully, we will have enough time at the end to have questions to the panel as a whole.

So, our first speaker is Miss Mary Schluckebier, who is the executive director of the Celiac Sprue Association, and I hope I pronounced that close to the way you like it pronounced.

Consumer Perspective on a Gluten-Free Food Labeling Standard

MS. SCHLUCKEBIER: Good afternoon. I want to thank you again for the opportunity to be here. I am Mary Schluckebier from the Celiac Sprue Association, and I think it is really kind of an interesting opportunity to get to share with you some of the things that are happening with our members, or what our members share with us 24 hours a day sometimes. It is very nice to have answering machines, and e-mail and calls. There are just a lot of people being diagnosed now that have more questions, and those are the ones really asking the questions of the manufacturers so that they are now looking to help us by ways of communicating with

each other.

At time of diagnosis--Dr. Hamilton did a very good job talking about the diagnosis and all that stuff as kind of a clinical thing. You have to think about it as this is a person. You have been eating a normal diet for years and they tell you these couple of things, first of all, about the disease itself; that there is no known threshold at this point that will create an immune response in people with celiac disease. There is no way to measure the presence of the offending amino acid sequences, and it shows in research that if you don't adhere to a strict diet you are going to get sicker--you know, that whole list of all the complications that can happen.

So, then you are told eliminate all foods and medications that are derived from wheat, barley, rye and oats at this time--I am going to abbreviate these as WBRO from now on--any of these foods that have the amino acids that evoke an immune response in people with celiac disease, which is what we mean. We mean that whole basket

but we are going to call it WBRO. For the remainder of your life do this and then you will be healthy.

That is exactly what happens for most people. After a few weeks on a gluten-free diet people are feeling really great and then they get into something that has wheat in it, and they want to avoid this if at all possible. Now that people are taking less than 11 years to be diagnosed, we are finding people who have basically no symptoms at all when they consume some wheat, rye, barley or oats.

That makes it even a little more confusing and they begin to rely more on information from food manufacturers. So, again, from this time on what you put in your mouth is your responsibility and the consequences are really up to you, and how successful you are in reading labels, gathering information and making your decisions.

I would like to say that all of us process this information. We don't All of us who have celiac disease set up some type of way we make a

decision multiple times a day on whether we are going to consume a product or not consume a product. Most people will seek information of one kind. Now, knowing that all information is not equal and that we all can't absorb everything the first week you are diagnosed and that it is a life-long disease, your decision-making process changes over the years.

But you seek out information and you have coping techniques. So, people with celiac disease often have a very broad-based knowledge about the disease and food. As a support group, we do have some help in helping people learn to do a self-management guide which has three stages. The initial stage is really looking at what you are eating right now and how can that be adapted to meet a gluten-free lifestyle so you don't have to make major changes immediately, and then slowly adding and evaluating foods that you want to change and adapt your diet and expand it to greater and greater food choices.

One of the things that we have

published--we are now getting ready for the tenth edition--is a 375-page resource for people with celiac disease where manufacturers have given us permission to put in print their products that they consider to be gluten-free or they consider appropriate for people with celiac disease and dermatitis herpetiformis.

We do spot-check some of these and have an ELISA test done on them, usually at 10 parts per million. Most companies' products always test below the level of detection of these. There are a few that do not, and a few that have surprised us, like beer. We tested three kinds of lite beer with an ELISA at 10 parts per million and they all came out below level of detection. We tested another product that had barley as a second ingredient, and the company gave us the test and it had been tested with one of the older ELISAs that was only sensitive for the gluten. So, we tested it at 3 parts per million in one that was cross-reactive; 3 parts per million barley, the second ingredient, below level of detection.

We are not sure why for some of these things, but what we do is we take the information back confidentially to the manufacturer and see if they can help us understand it. Often then they make changes that make it more appropriate for people with celiac disease if it is over, or they feel great because they are doing what they think they are doing.

To make an informed decision you need to know what is present in a product and where it really came from. Natural flavorings has been a problem for people with allergies and celiac disease, and it is one of the really good examples though it is really hard to know, especially since barley is a very common flavor enhancer.

So, in a person with celiac disease the ingredient list gives you a good idea of what is in the product. Calling the manufacturer on processing and packaging is sometimes necessary if, in your decision-making process, when you have tried the product something isn't working and you are trying to eliminate which product of all the

20-something you ate during the last little bit. Then you often call the manufacturer and find out a little bit more about the product's processing and packaging and see if there is any other way that this could be cross contaminated, or if you can eliminate that this is not the problem food.

But when you eat 20 or 30 things a day--just think of how many things you eat--trying to figure out which one created a problem--now, we are doing this on symptoms, and all of you know that symptoms are not a good indicator of what is going on and they are very misleading. The symptom really may be that I am catching a cold so I am reacting to something that may be totally unrelated--maybe catching a cold isn't a good idea.

So, people use their decision-making process and try and ferret out what is good for their diet. That is why this meaning, full verifiable and consistent, is really what we are trying to pull together, and this meaningful thing is when I say gluten-free it can mean so many things to all of us. I am glad we use the

definition--you know, we kind of came up with definitions of how we are going to talk today but when I am newly diagnosed and somebody says you are giving up gluten and you have never even heard of gluten, it is really hard and there is a learning curve. Again, as consumers, often our manufacturer consumer service groups have been the educators of our members.

Verifiable--we were talking again about the ELISA or being consistent. So, when I see gluten-free on one label it should mean the same as everything else. And, generally on gluten-free our members said they want it to mean the absence of wheat, barley, rye and oats and that whole basket of other things. But generally if you have any of those other items on a food label they are going to be in the label because they are a selling point and a marketing point for the manufacturers.

These are some of the things that people develop in their decision-making process. The cross contact--now, again, that can go from the field to the fork and often, as a home economist, I

watch people prepare something and it often happens right there in the kitchen. It happens when somebody dips into the peanut butter jar and puts it on the bread and goes in again and then gives it to the person who is trying to avoid a wheat product. It is very difficult to explain to people that a person with celiac disease may have to watch and be cognizant of all these potentials for their entire consuming life. And, I am thinking why I gained weight. Sometimes I wonder if we just shouldn't eat just a few things and then there would be a lot less to figure out and we wouldn't gain weight, and it would be a weight loss program and it would be easier to sell.

The cross contact begins at the farm, and I am a farmer. This was near our farm. Harvesting of summer wheat, spring wheat, winter wheat and oats often in the Midwest is within two or three weeks of each other. They are always going out to harvest just before a rain because it is coming, and you have to get it out and you know if you let it go--so it is always under pressure. I have been

married 30-some years and it is always under pressure. We don't do this anymore. We don't do the wheat anymore. But this one is near our farm. There is no fence. There is no buffer zone. And there is not, at this time, any regulation that would call for that. But here the wheat has already been cut. If you notice, there is a green tinge to the oats. It is not quite ready but there is wheat growing in the oats and oats growing in the wheat. But we don't have to worry about cross pollination because these are not cross pollinating crops but they are harvested and handled in much the same manner and through the same harvesting equipment, and they are a consideration.

Now, I said my husband used to be a wheat grower. We don't do wheat anymore. But there is a quality control that I think about applying--olives, the olives for the olive oil, they go through and they separate them by grades. Maybe we just need a few more grades so that a pharmaceutical grade would be adequate for people with allergies and gluten-free. As a farmer, I

think this kind of sounds good because we maybe could get more money for our crops. But right now it is not even a near possibility.

So, when we ask our members across the country about oats, right now eliminating oats from their diet, 151 said they ate it and 22 had no answer to the question. When we asked how would you like to have gluten-free defined, the first time we asked that a few years ago we said so many parts per million and gave them checks. They basically said, "don't tell me the parts per million, I want it absent of wheat, barley, rye and oats. I don't know what these numbers mean."

I think there is a potential for oats here. It shows that there are people who really don't know what it is and are eating. It is totally inconclusive on oats from everything I have read. When I became a celiac in 1986 they were questioning oats, whether or not to have oats. We had 1,101 define gluten-free as the absence of WBRO and 77 others who said they would like to see it that way but they don't know whether oats should be

included or not be included in the definition.

At the threshold meeting, Dr. Colin presented the fact that in the study that they did 10 percent of the participants with CD did get immune symptoms and 19 percent of DH, and I just think that maybe we need to look into this subset and see if it is a real subset of people with celiac disease who can or cannot ingest oats without any immune response.

On packaging, if it could be that gluten-free means the absence of all wheat products in the product itself and then be verified with testing, we would avoid things like this. Both of these gravy mixes are marked gluten-free. One has hydrolyzed wheat protein, and then it says ELISA tested and gluten is removed, There is no standardization for any of this and it is confusing for a consumer.

The other package has rye malt extract as a seventh ingredient, again, you saw gluten-free on the other side. We had this one tested at the University of Nebraska with the RidaScreen and it

tested 3,630 parts per million and we were talking 10 and 3 earlier. When we have tested things there isn't a real consistency, and I wish there were.

One of the things is we have to have all the ingredients on the product label. This is candied walnuts. This one has wheat starch in with the walnuts. Some of the food processing people can help me a little bit with this, but I think wheat starch is one of the wonderful coatings you can use to help keep things fresh. That also then creates a problem for a person with celiac disease. They learn to read labels even when you think you don't need to read labels. One manufacturer said since I started working with people with celiac disease I have learned about a new technique. Celiacs pick up a package and give it the celiac twist, they turn it and look at the ingredients. If you want to know anything about any ingredient you would probably ask the person with celiac disease. They probably know that food and nutrition label better than anyone else because they have practiced a lot.

Also things that are a little confusing on labels are wheat fiber that is certified gluten-free and we don't know what the certification program for it is. Wheat grass, again, wheat grass has not been really tested because the people who test wheat test it as it is emerging and then as it is a mature crop, and as a grass there is no research. Hydrolyzed wheat protein, again, is one of those where the ELISA test is not the most effective way of recognizing it. So, this is an area that requires a little more research before we really know how to even deal with these. So, again, a definition that just says the absence of anything from wheat, barley, rye and oats is probably the most conservative at this point.

When we ask manufacturers whether they have these ingredients in their products, "well gluten is not present in oats and barley because we use only the outer portion of the grain kernel and that is gluten-free." "Wheat fiber is certified to be gluten-free." "We adhere to the Codex

definition of gluten-free." "And it tested below the level of detection for gliadin so it is gluten-free." When they were talking about the testing, there are some that are just specific for gliadin and some that do a crossover for the other prolamins.

If you want to know what our group looks like that were in the last two surveys, we are mostly women. We are over 45 that stay members of CSA. One of the things that happens with membership in an organization like a support group is that as soon as people become very comfortable in their decision-making process they are like butterflies and they fly off and go do their own, and feel like they can manage their own disease, which is good but it means that often we don't have everybody who is a celiac as part of our organizations. In Europe, if you are diagnosed you automatically become a member of organizations, such as in England.

This is the one that always surprises me. When you ask the educational level, the education

level of our members--those last three columns--is some college, college and postgraduate. I don't know if it takes somebody really smart to get diagnosed or, like one of my medical advisers said, you can't make any kind of claim on those. But it does mean that we do have people who are readily reading labels. But we also have people who are celiacs that are blind, they can't hear, and we have those who have below 100 in mental capacity, and we have had social workers call and say, "how am I going to protect the child that has a mother who is not functional in the grocery store and at reading labels?" The one to the right is to show you that most people who have celiac disease are just normal people with all the other problems that we can have, including other food concerns and you will notice that milk is the most common. When we ask how many food concerns do you have, or allergies, or whatever, it was real interesting that almost half of us have something else we are looking at that label for. So, just marking gluten-free, if this is indicative and you can use

this small group to predict the larger group, there will be at least half the people who have to read the label for something else.

But when we ask them what do you consider your greatest challenge as a person with celiac disease, the top one, overwhelmingly the top one is lack of good food labeling. Now, I don't know how you evaluate this part but they put avoiding cross contamination along with concerns with traveling and making decisions when you are eating out. They weigh both about the same.

Where do you go to purchase your products? People overwhelmingly say health food stores, followed by grocery stores, mail order, internet and super stores. When we ask them also where do you think other people with celiac disease go to buy their foods--grocery stores.

We ask people to rate themselves with how sensitive they thought they were to food, medications, the skin, contact of medications, hair products, detergents and lotions and soaps, you will see that it is kind of all over the place.

People all say that their food concerns are the highest. They are most sensitive to food. But notice that there are people from very highly sensitive to those that are hardly sensitive at all.

Then we ask them how will you rate your risk of choosing a product if you have incomplete information. Will you take a risk? What is your risk management technique? No matter where they were on that sensitivity curve, we don't repeat the same one here. It is almost overwhelmingly that they are adverse to risk. They will take as little risk as they need to, to continue on their diet.

So, what else do you look at when you are looking at a label to purchase something? First of all, most people are going to look at the ingredients even if there is a marking that says gluten-free. Then, after that, will they buy a product with a seal or something that says it is gluten-free? Most people will buy it at least once.

This is what we are doing as part of our